Determination of Long-Chain Hydroxamic Acids

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A procedure for determining long-chain hydroxamic acids consists in hydrolysis to carboxylic acid and hydroxylamine hydrochloride with a known excess of aqueous, alcoholic hydrochloric acid, followed by titration of either the excess hydrochloric acid or the hydroxylamine hydrochloride formed. The former technique gives slightly low results; the latter, slightly high. Hydroxylamine hydrochloride cannot be titrated in the presence of fatty acids containing ten or less carbon atoms.

IN A study of the reaction of glycerides and other esters of long-chain fatty acids with hydroxylamine, it was necessary to determine hydroxamic acids in the presence of unconverted esters in order to calculate the degree of conversion, and also to determine the purity of the hydroxamic acids isolated by crystallization. A method was desired which was rapid and accurate, did not require specialized apparatus, and could be employed as a routine method.

Direct titration of hydroxamic acids with alkali using phenolphthalein as indicator (*7*) was unsatisfactory because of the extremely low acid strength of long-chain hydroxamic acids. Reaction of hydroxamic acids with bromine (*6*) could not be employed because of interference by double bonds. Bromic acid oxidation (*4*) was unsatisfactory because of the difficulty in obtaining a suitable reaction system for the water-insoluble hydroxamic acids and aqueous potassium bromate (the source of bromic acid). Reaction systems containing ethyl alcohol, acetic acid, chloroform, and dioxane as cosolvents were investigated. It was thought that a reliable iodine number could be obtained on unsaturated hydroxamic acids, and the bromine reaction method (*6*) could be corrected for absorption of bromine by the double bonds present. This failed because even pure saturated hydroxamic

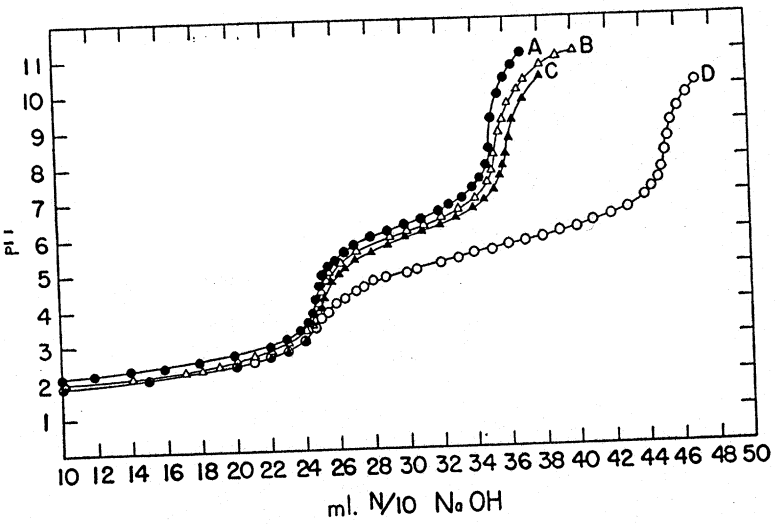
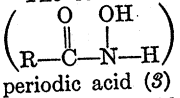


Figure 1. Titration Curves of Synthetic Mixtures

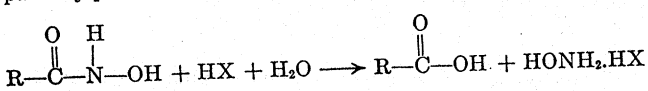
A. 0.00100 mole of hydroxylamine hydrochloride, 25 ml. of 0.1 *N* HCl, 50 ml. of H_2O
B. Same as *A* + 0.00106 mole of oleic acid
C. Same as *A* + 0.00100 mole of lauric acid + 10 ml. of 95% ethyl alcohol
D. Same as *A* + 0.00102 mole of caprylic acid + 10 ml. of 95% ethyl alcohol

acids consumed significant but variable quantities of Wijs solution.

The formal structural similarity between hydroxamic acids  and α-ketols suggested the possibility that periodic acid (*3*) might be a useful reagent for the analysis of hydroxamic acids. Investigation of the usual experimental variables (time, temperature, excess of periodic acid, etc.), as well as various solvent systems, indicated that the equivalent weight of the hydroxamic acids was approximately one third the molecular weight, when 80% acetic acid was employed as the solvent for the periodic acid and dioxane as the solvent for the hydroxamic acid. Results were erratic, however, and it was soon discovered that when peroxide-free dioxane was employed, no consumption of periodic acid occurred. It was demonstrated by a series of control experiments, in which dioxane-containing peroxide was employed but the periodic acid was omitted, that the peroxides in the dioxane were not reacting with the hydroxamic acids.

METHOD EMPLOYED

The observation by Inoue and Yukawa (*1*, *2*) that hydroxamic acids are quantitatively converted to the parent carboxylic acids by heating with dilute aqueous alcoholic sulfuric acid suggested that a simple hydrolytic reaction might be employed for the determination of hydroxamic acids. Because acid hydrolysis apparently proceeds according to the equation



is was evident that for each mole of hydroxamic acid hydrolyzed, one mole of hydroxylamine salt should be formed, consuming one mole of mineral acid. A simple approach, therefore, would be to employ a known excess of mineral acid in the hydrolysis, titrate unconsumed acid, and by difference obtain the quantity of mineral acid equivalent to hydroxylamine liberated, which in turn is equivalent to hydroxamic acid. Alternatively, by a differential technique it might also be possible to titrate liberated hydroxylamine salt directly. Consideration was also given to the direct determination of hydroxylamine liberated by the ferric-ferrous reaction, but it was eliminated from study because of the insolubility of iron salts of long-chain fatty acids.

It was necessary to determine whether free mineral acid and hydroxylamine salt could be determined in the presence of each other and free fatty acid.

In a control experiment, 0.0696 gram (0.00100 mole) of hydroxylamine hydrochloride, 0.2992 gram (0.00106 mole) of oleic acid, and 25 ml. of 0.1 *N* aqueous hydrochloric acid were mixed and titrated potentiometrically. At the first point of inflection in the titration curve, 24.8 ml. of 0.1 *N* aqueous sodium hydroxide had been consumed and at the second inflection an additional 10.4 ml. had been consumed. These titrations correspond to hydrochloric acid and hydroxylamine hydrochloride added, respectively (calculated, 25.0 and 10.0 ml.). Repetition of this experiment with synthetic mixtures containing equivalent quantities of myristic, lauric, capric, or caprylic acid instead of oleic acid gave satisfactory results in the titration of free hydrochloric acid in all cases (25.0 to 25.2 ml. of 0.1 *N* sodium hydroxide consumed), slightly high results (10.6 to 10.9 ml. of 0.1 *N* sodium hydroxide consumed) in the titration of hydroxylamine hydrochloride when lauric or myristic acid was present, and extremely high results in the titration of hydroxylamine hydrochloride in the presence of capric or caprylic acids. These last two compounds are stronger acids than the longer-chain acids and are titrated in large part before all of the hydroxylamine hydrochloride is neutralized. An additional control mixture containing only the hydrochloric acid and hydroxylamine hydrochloride was also titrated.

The titration curves for this mixture and three other mixtures containing a fatty acid are shown in Figure 1.

A systematic study was made of conditions necessary to achieve complete hydrolysis of hydroxamic acids and the following procedures given in detail were adopted.

HYDROXAMIC ACID ANALYSIS (ACID HYDROLYSIS)

Reagents. Ethyl alcohol, U.S.P., 95%. Hydrochloric acid of normality preferably between 0.9 and 1.0 *N*. Sodium hydroxide, 0.1 *N*.

Procedure. Weigh sample into small glass weighing cup, in accordance with the accompanying table, and place in special iodine flask with side arms to accommodate electrodes (*5*). (When the sample is known to contain less than 100% hydroxamic acid, its weight is increased proportionally.)

Approximate Equivalent Weight of Hydroxamic Acid	Weight of Sample
300	0.45–0.55
270	0.40–0.50
240	0.35–0.45
215	0.30–0.40
190	0.30–0.35
160	0.25–0.30

Add exactly 5 ml. of 1 *N* hydrochloric acid (a 200% excess of hydrochloric acid is employed) from a pipet and 10 ml. of 95% ethyl alcohol. Reflux the sample on the steam bath for 2 hours (4-hour reflux time is suggested when hydroxamic acid content is below 50%) and wash the stoppers and sides of the flask with 50 ml. of distilled water.

METHOD I. Titrate with 0.1 *N* sodium hydroxide to a pH of 4, using a pH meter with external electrodes. Run blank determinations.

$$\text{Hydroxamic acid, \%} = \frac{A \times N \times M.E.}{\text{weight of sample}} \times 100$$

where A = difference between blank and sample titration, ml.
N = normality of sodium hydroxide
$M.E.$ = milliequivalent weight of hydroxamic acid

$$\text{Equivalent weight of hydroxamic acid} = \frac{(\text{weight of sample})(1000)}{A \times N}$$

METHOD II. When it is known that acids containing ten or less carbon atoms are absent, hydroxylamine hydrochloride can be titrated, instead of or in addition to unconsumed hydrochloric acid, although higher results must be anticipated. The difference between titration to pH 4 and pH 8 is equivalent to hydroxylamine hydrochloride and to hydroxamic acid originally

present. The calculations shown above are used, except that A is the difference between the titrations at pH 4 and pH 8.

Several indicators were tried but were found unsatisfactory, probably because the change in pH was not sufficiently abrupt to give a distinct color change.

RESULTS

Table I shows the results obtained in the analysis of pure and crude hydroxamic acids, some synthetic mixtures, and a reaction product obtained from tallow and hydroxylamine.

The results obtained by Method I are usually a few percentage units low; those by Method II are a few percentage units high, except when acids containing ten or less carbon atoms are formed. Either method of titration can be used when the chain length of the hydroxamic acids is in the proper range, although Method I is more convenient because only one end point is required and knowledge of the chain length is not necessary. Although the absolute accuracy of the method is not so good as is usually desired, the results are sufficiently accurate for most purposes. Precision of duplicate determinations is usually within 1%. The main advantage of the method is its simplicity.

ACKNOWLEDGMENT

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Table I. Hydroxamic Acid Analyses

Material Analyzed	Hydroxamic Acid Found, % Method I[a]	Method II[b]
Oleohydroxamic acid, m.p. 64.1–64.7° [c,d]	95.9–98.5	99.2–103.2
Stearohydroxamic acid, m.p. 106.2–106.8° [c,e]	95.3–96.9	101.3–101.5
Caprylohydroxamic acid, m.p. 77.9–78.4° [c,f]	95.0–96.8	135–195
Stearohydroxamic acid, crude reaction product[c]	76.2–79.2	..
Oleohydroxamic acid, crude reaction product[c]	81.7–82.0	..
Oleohydroxamic acid, 77.2%; beef tallow, 22.8%	71.0	80.8
Oleohydroxamic acid, 60.2%; beef tallow, 39.8%	53.3	62.4
Oleohydroxamic acid, 0%; beef tallow, 100%	0–0.6	0–5.8
Stearohydroxamic acid, 75.0%; methyl stearate, 25.0%	68.1	72.6
Stearohydroxamic acid, 25.0%; methyl stearate, 75.0%	24[g]	31.1[g]
Reaction product of tallow with hydroxylamine[h]	50.1	..

[a] Difference between blank and back-titration to pH 4 employed in calculation.
[b] Difference between titrations at pH 4 and 8 employed in calculation.
[c] Prepared by mixing a solution of 0.1 mole of the methyl ester of the corresponding carboxylic acid in 180 ml. of absolute alcohol with a solution of 0.15 mole of hydroxylamine hydrochloride and 0.250 mole of NaOH in 180 ml. of absolute alcohol, and refluxing for 1 hour. Dilute aqueous HCl was then added to the cooled reaction mixture until the pH was 4 and the mixture was diluted with a large volume of water. The crude reaction product was filtered, washed free of salt, and dried. It was recrystallized from petroleum naphtha, hexane fraction, boiling range 63° to 70° (10 to 15 ml. of solvent per gram of solute) at 0° to constant melting point.
[d] Calcd. C, 72.7; H, 11.9; N, 4.71. Found. C, 72.8; H, 11.7; N, 4.80.
[e] Calcd. C, 72.2; H, 12.5; N, 4.68. Found. C, 72.1; H, 12.4; N, 4.73.
[f] Calcd. C, 60.3; H, 10.8; N, 8.80. Found. C, 60.3; H, 10.7; N, 8.74.
[g] 4-hour reflux time during analysis.
[h] Prepared as described in [c], but 0.03 mole of tallow used instead of 0.1 mole of methyl ester.

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